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Camici, Giovanni G ; Savarese, Gianluigi ; Akhmedov, Alexander ; Lüscher, Thomas F

**Abstract:** Western societies are aging due to an increasing life span, decreased birth rates, and improving social and health conditions. On the other hand, the prevalence of cardiovascular (CV) and cerebrovascular (CBV) diseases rises with age. Thus, in view of the ongoing aging pandemic, it is appropriate to better understand the molecular pathways of aging as well as age-associated CV and CBV diseases. Oxidative stress contributes to aging of organs and the whole body by an accumulation of reactive oxygen species promoting oxidative damage. Indeed, increased oxidative stress produced in the mitochondria and cytosol of heart and brain is a common denominator to almost all CV and CBV diseases. The mitochondrial adaptor protein p66Shc and the family of deacetylase enzymes, the sirtuins, regulate the aging process, determine lifespan of many species and are involved in CV diseases. GDF11, a member of TGF $\beta$  superfamily with homology to myostatin also retards the aging process via yet unknown mechanisms. Recent evidence points towards a promising role of this novel 'rejuvenation' factor in reducing age-related heart disease. Finally, telomere length is also involved in aging and the development of age-related CV dysfunction. This review focuses on the latest scientific advances in understanding age-related changes of the CV and CBV system, as well as delineating potential novel therapeutic targets derived from aging research for CV and CBV diseases

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**Translational medicine**

# Molecular mechanism of endothelial and vascular aging: implications for cardiovascular disease

**Giovanni G. Camici<sup>1\*</sup>, Gianluigi Savarese<sup>2</sup>, Alexander Akhmedov<sup>1</sup>, and Thomas F. Lüscher<sup>1,3</sup>**

<sup>1</sup>Center for Molecular Cardiology, University of Zurich, Zurich, Switzerland; <sup>2</sup>Cardiology Unit, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden; and <sup>3</sup>University Heart Center, Cardiology, University Hospital Zurich, Zurich, Switzerland

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Western societies are aging due to an increasing life span, decreased birth rates, and improving social and health conditions. On the other hand, the prevalence of cardiovascular (CV) and cerebrovascular (CBV) diseases rises with age. Thus, in view of the ongoing aging pandemic, it is appropriate to better understand the molecular pathways of aging as well as age-associated CV and CBV diseases. Oxidative stress contributes to aging of organs and the whole body by an accumulation of reactive oxygen species promoting oxidative damage. Indeed, increased oxidative stress produced in the mitochondria and cytosol of heart and brain is a common denominator to almost all CV and CBV diseases. The mitochondrial adaptor protein p66<sup>Shc</sup> and the family of deacetylase enzymes, the sirtuins, regulate the aging process, determine lifespan of many species and are involved in CV diseases. GDF11, a member of TGF $\beta$  superfamily with homology to myostatin also retards the aging process via yet unknown mechanisms. Recent evidence points towards a promising role of this novel 'rejuvenation' factor in reducing age-related heart disease. Finally, telomere length is also involved in aging and the development of age-related CV dysfunction. This review focuses on the latest scientific advances in understanding age-related changes of the CV and CBV system, as well as delineating potential novel therapeutic targets derived from aging research for CV and CBV diseases.

**Keywords** Aging • p66<sup>Shc</sup> • JunD • Sirtuins • GDF11 • Cerebrovascular • Cardiovascular

## Introduction

Aging is a major risk factor for the occurrence of acute and chronic cerebrovascular (CBV) and cardiovascular (CV) diseases, such as stroke and myocardial infarction. Indeed, in currently used risk scores such as the one of the *European Society of Cardiology*,<sup>1</sup> the weight of age surpasses that of any known CV risk factor. Of note, visible age-related signs such as male pattern baldness, grey hair, facial wrinkles as well as the presence of arcus corneae alone or in combination with appearance factors such as earlobe crease and xanthelasma provide additional risk prediction beyond known CV risk factors.<sup>2</sup>

According to the World Health Organization, the European population is projected to increase only slightly by 2020, from 894 to 910 million, but then to return to current levels by 2050.<sup>3</sup> As a consequence, the number of elderly people is expected to rise substantially. Indeed, the number of individuals aged 85 years and older is projected to increase from 14 to 19 million by 2020 and to 40

million by 2050.<sup>3</sup> The underlying process of global population aging has been named 'demographic transition' in which both mortality and fertility decline. Decreasing fertility together with improving social and health conditions determine a further increase in lifespan and play a key role in the aging pandemic that characterizes the 21st century.<sup>4</sup>

Cardiovascular and CBV diseases are age-related pathologies. Of three adults, at least one suffers from CV or CBV disease and more than half of those are estimated to be over 60 years of age. Of note, the average annual rate of a first CV or CBV event rises from 3 per 1000 for men at 35–44 years of age to 74 per 1000 for those at 85–94 years of age. For women, comparable rates occur 10 years later in life.<sup>5–7</sup>

According to the latest data, only 30% of deaths in people aged under 65 are caused by CV and CBV diseases, as are 37% of deaths occurring before age 35; this means that more than half of deaths related to CV and CBV diseases develops in individuals aged 65–74 years.<sup>8</sup>

These alarming data urge for research focussing on the interconnections between aging, CV, and CBV diseases in order to discover novel therapeutic targets allowing to address the constantly increasing clinical needs in elderly patients.

## Theories of aging

### Free radical theory of aging

One of the most accepted theories of the mechanism of aging is the free radicals theory first conceived by Harman.<sup>9</sup> It relies on the concept that reactive oxygen species (ROS) produced during normal aerobic metabolism tend to accumulate with age ultimately resulting in oxidative damage of genomic DNA, proteins, and cellular components.<sup>9</sup> According to this theory, an increase in pro-oxidants would promote, whereas an improvement in antioxidant defences would delay the aging process.<sup>9</sup> The relevance of this hypothesis has been verified *in vitro* and in animal models by modulating genes directly involved in the metabolism of ROS, such as superoxide ( $O_2^-$ ) dismutase that converts  $O_2^-$  to hydrogen peroxide and then to water; catalase that converts hydrogen peroxide to water and oxygen; glutathione peroxidase that reduces lipid hydrogen peroxides to their corresponding alcohols and free hydrogen peroxide to water; thioredoxins that facilitate reduction of other proteins via their dithiol-disulphide active site and detoxify peroxides; peroxiredoxins that reduce hydrogen peroxides and peroxynitrite; and methionine sulfoxide reductases that carry out the enzymatic reduction of methionine sulfoxide to methionine.<sup>10–14</sup>

Of note, reduced levels of these protective proteins are associated with increased morbidity and reduced lifespan.<sup>10–14</sup> In conditions of elevated oxidative stress,  $O_2^-$  binds nitric oxide (NO) resulting in the generation of peroxynitrite ( $ONOO^-$ ), another highly reactive and toxic species which penetrates across the phospholipid membrane causing substrate nitration damaging DNA,<sup>15</sup> oxidation of lipoproteins,<sup>16</sup> disruption of mitochondrial activities,<sup>17</sup> nitrosylation of proteins, and further depletion of plasma antioxidants.<sup>18</sup>

Nitric oxide is the most important regulator of the CV system.<sup>19</sup> When the release of NO or its bioavailability is reduced, endothelium-derived contracting factors become predominant, determining the onset of endothelial dysfunction,<sup>20</sup> the common denominator of hypertension, diabetes, and hypercholesterolemia.<sup>21</sup> Reduced NO bioavailability endorses adhesion of platelets and leukocytes, as well as migration and proliferation of smooth muscle cells which determine the first stages of atherosclerosis.<sup>20</sup> The activation of other factors induced by ROS, such as the transcription factor NF- $\kappa$ B, further promotes atherogenesis by the release of several cytokines, such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 6, monocyte chemoattractant protein-1, and adhesion molecules, which collectively contribute to chronic inflammation, a main determinant of atherosclerosis.<sup>22</sup>

The renin–angiotensin–aldosterone system (RAAS) is implicated in aging by increasing tissue and mitochondrial oxidative stress.<sup>23</sup> Angiotensin II (Ang II), the key effector molecule of RAAS, activates via its receptor type 1 NADPH oxidase, which in turn generates superoxide anion ( $O_2^-$ ).  $O_2^-$  promotes then uncoupling of endothelial NO synthase, followed by impairment in NO bioavailability

and finally leads to enhanced ROS production. Activation of Ang II and its consequent effects on free radical production are tightly controlled under physiological conditions. Therefore, uncontrolled Ang II-mediated ROS generation takes place as a result of age-dependent RAAS activation.<sup>24</sup> In addition, Ang II also accelerates cellular senescence by telomere shortening.<sup>25</sup>

### Telomere shortening theory of aging

Telomeres are a region of repetitive nucleotide sequences (TTAGGG) found at the ends of chromosomes. They are considered to have several functions, including protection against degeneration, reconstruction, fusion and loss, as well as contributing to pairing of homologous chromosomes. Telomeres are shortened by 33–120 base pairs with each cell division, up to a critical length which induces the loss of the complex nucleoprotein structure, thereby triggering replicative senescence—a permanent non-dividing state which ensues in somatic cells after a predetermined number of cell divisions.<sup>26</sup> Telomerase is an enzyme responsible for maintaining the length of telomeres and it is highly expressed in over 90% of cancers. It is believed that its upregulation is a crucial mechanism for the avoidance of cellular senescence. Telomerase-deficient mice are susceptible to progressive tissue atrophy, stem cell depletion, organ system failure, and impaired tissue injury responses, which are reversed by telomerase reactivation.<sup>27</sup> This suggests that telomere shortening is the main determinant of cellular aging. In addition, some associations between shorter telomeres and CV diseases such as calcific aortic valve stenosis, atherosclerosis, and myocardial infarction have been shown over last decade.<sup>28–30</sup> However, some concerns about this theory need to be acknowledged. First of all, although telomere shortening may play a role in determining lifespan in cells that continue to divide, it seems unlikely to contribute to the aging process that occurs in postmitotic cells. Additionally, the aging process is not limited to dividing cells. In fact, several human cells, such as muscle cells (i.e. the heart among other organs) or central nervous system neurons, do not divide during adulthood, yet show progressive morphological signs of aging during senescence. Of note, the correlation between telomere length and age-related disorders is still a matter of debate.<sup>31</sup> Whereas some prospective studies showed association between short telomeres and overall mortality,<sup>32–34</sup> other studies did not find the same association.<sup>35–37</sup> The role of telomere shortening in cancer, which can also be considered an age-related disease since its probability increases with age, is still not clear. Although numerous studies investigated the link between telomere length in malignant cells and cancer progression or survival, this relationship still remains insufficiently understood and requires further clarification.<sup>38,39</sup>

Therefore, despite existing evidence associating telomere length and age-related diseases, neither conclusive causative link nor a predictable correlation can be postulated.

## Genes regulating aging and longevity

### The histone deacetylases sirtuins

Sirtuins belong to class III histone deacetylases and have mono-ADP-ribosyltransferase, lysine deacetylase, desuccinylase,

depalmitoylase, demalonylase, and demyristoylase activity. Their nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent enzymatic activity has been associated to aging and it could represent an interesting mediator of aging and age-associated CV/CBV diseases.<sup>40–42</sup>

Seven sirtuins which have different cellular localizations have been reported in mammals: Sirt1, that is localized in the nucleus and in the cytoplasm, is implicated in the control of cell survival and metabolism; Sirt2, localized in the cytoplasm, regulates cytoskeletal reorganization, autophagy and metabolism; Sirt3, Sirt4 and Sirt5 are located in the mitochondrion and regulate ROS production, apoptosis, and metabolism; Sirt6, localized in the nucleus, regulates gene expression, cellular metabolism, and inflammatory response; Sirt7, that is also in the nucleus, is implicated in gene transcription.<sup>43</sup>

Several members of sirtuin family have been demonstrated to play a key role in human aging. Sirt1 endogenous expression has been studied in young and old human donors of vascular smooth muscle cells (VSMCs) (from 12- to 88-year-old subjects), demonstrating an inverse correlation between the endogenous Sirt1 protein expression and the donor age.<sup>44</sup> In particular, a trend toward reduced mortality has been shown for Sirt1 haplotype 2 and rs3758391 single-nucleotide polymorphism (SNP) carriers among the 1245 participants of the Leiden 85-plus study,<sup>45</sup> while a significant 31% risk reduction was observed for the rs12778366 SNP carriers among the 1390 subjects of the Vlagtwedde/Vlaardingen cohort.<sup>46</sup> Several reports speculate also on the involvement of Sirt3 in determining lifespan. Despite no significant difference in Sirt3 mRNA or total Sirt3 protein expression of myocardial tissues was observed between young and old patient groups, protein expression of the short isoform of Sirt3 (sh-Sirt3) was significantly increased in young tissue vs. old tissue, whereas the expression of the full-length Sirt3 (fl-Sirt3) was higher in the aged vs. the young group.<sup>47</sup> Several studies suggest that Sirt3 genetic variability might be relevant for the modulation of human longevity. In particular a silent G/T transversion at position 477 of the coding region (G477T corresponding to Ser159Ser; AF083108) of Sirt3 gene was observed in an Italian cohort from Calabria (801 subjects free of clinically overt pathologies, 120 older than 100 years), with an improved survival in males with TT genotype and a higher mortality in males with GT genotype.<sup>48</sup> Similarly, Sirt3 rs11555236 and rs4980329 SNP carriers have been shown to be associated with an improved survival in females in another Italian cohort from Treviso.<sup>49</sup> A recent study focused on the role of Sirt3, Sirt5, and Sirt6 on human aging.<sup>50</sup> It demonstrated in 3763 subjects that homozygous minor allele genotypes within rs2841505 (Sirt5) and rs107251 (Sirt6) are associated with reduced survival, whereas homozygous minor allele genotypes within rs511744 (Sirt3) determined an increased lifespan.

The role of sirtuins in age-related CV/BCV diseases has been deeply investigated. Sirt1 is considered an interesting target to delay vascular aging through its antioxidant properties. In fact, it increases the activity of catalase and induces manganese superoxide dismutase, two key enzymes involved in controlling cellular ROS levels, by deacetylation of the mammalian forkhead transcription factors of the O class in response to oxidative stress.<sup>51</sup>

Additionally, Sirt1 expression and activity gradually decrease with aging and in parallel to this, oxidative stress, which is a major cause of atherosclerosis, increases.<sup>52,53</sup> The protective role of Sirt1 in

atherosclerosis has been demonstrated in *Sirt1-Tg/ApoE<sup>-/-</sup>* mice which have shown reduced atherosclerotic plaque formation when compared with *ApoE<sup>-/-</sup>* following 10 weeks of high-fat diet. Furthermore, in similar experiments mice overexpressing endothelial cell-specific Sirt1 have maintained acetylcholine-induced relaxations of the aorta accompanied by upregulated endothelial NO synthase (eNOS) thus confirming that Sirt1 modulates eNOS expression and activity.<sup>54</sup> On the other hand, *Sirt1<sup>-/-</sup>/ApoE<sup>-/-</sup>* mice exhibit enhanced plaque formation.<sup>55</sup> This suggests that during high-cholesterol diet, Sirt1 suppresses atherogenesis by maintaining endothelial cell survival and function. In a recent study, *ApoE<sup>-/-</sup>* mice fed for 12 weeks with a high-cholesterol diet supplemented with SRT3025, a pharmacological Sirt1 activator, showed reduced plasma levels of LDL and total cholesterol as well as reduced plaque formation. Considering that SRT3025 reduced the hepatic release of acetylated PCSK9 and in turn increased the expression of LDL-receptors and reduced plasma levels of LDL in *ApoE<sup>-/-</sup>*, but not *LDL-R<sup>-/-</sup>* mice reduction in PCSK9 appears the most likely mechanism by which Sirt1 blunts atherosclerosis.<sup>56</sup>

Low levels of Sirt1 have been reported also in circulating peripheral blood mononuclear cells of patients with metabolic syndrome.<sup>57</sup> In mice, Sirt1 overexpression is associated with reduced levels of serum insulin and cholesterol together with a reduction in adipose tissue volume and a decreased obesity-induced insulin resistance.<sup>58,59</sup> Additionally, Sirt1 could also play a role in the regulation of whole-body metabolic homeostasis.<sup>60</sup> Other members of the Sirtuin family also affect glucose metabolism. In particular, Sirt3 increases insulin sensitivity and decreases serum glucose,<sup>61</sup> while Sirt4 inhibits glutamate dehydrogenase, which converts glutamate to  $\alpha$ -ketoglutarate in the mitochondrion, repressing amino acid induced insulin secretion.<sup>62</sup>

Sirt1 together with Sirt3 and Sirt6 modulate cardiac hypertrophy by regulating AKT, a gene that plays a central role in regulating a variety of cellular processes ranging from cell survival to aging.<sup>63</sup> Indeed, left ventricular hypertrophy represents a predictor of outcome, since it lowers coronary reserve and enhances cardiac oxygen requirements.

Furthermore, it appears that sirtuins are also involved in coronary artery disease (CAD). Indeed, levels of Sirt1 mRNA have been evaluated in peripheral monocytes of 48 male subjects admitted for cardiac catheterization and subdivided into individuals with normal coronary arteries, patients with stable CAD and those with acute coronary syndromes (ACS). Of note, Sirt1 levels were reduced in patients with stable CAD and in those with ACS when compared with those without angiographically demonstrable CAD. Interestingly, Sirt1 levels correlated positively with HDL levels in all groups. In line with this observation, THP-1 monocytic cells incubated with HDL isolated from healthy subjects displayed increased Sirt1 protein expression when compared with cells incubated with HDL from the CAD or ACS patients. Furthermore, PON1 activity, an important antioxidant enzyme assuring proper biological activity of HDL,<sup>64</sup> was reduced in HDL from patients with CAD or ACS, indicating that PON1 activity is required to allow HDL to stimulate Sirt1 expression.<sup>65</sup>

Taken together, sirtuins are potentially interesting therapeutical targets for the treatment of age-related CV disease. In particular, Sirt1 affects oxidative stress, metabolic syndrome, diabetes mellitus,

cardiac hypertrophy, and atherosclerosis. The development of molecules stimulating Sirt1 expression and activity supports this concept. To date several natural and synthetic Sirt1-activating compounds have been described.<sup>66,67</sup> Natural plant-derived metabolites able to activate Sirt1 *in vitro* include flavones, chalcones, anthocyanidins, and resveratrol that has been demonstrated to be the most promising for therapeutical purposes.<sup>66</sup> The first synthetic Sirt1-activating compounds to be produced were chemically different from resveratrol and presented an imidazothiazole scaffold (SRT1460, SRT1720);<sup>68</sup> recently, a more potent second generation of synthetic Sirt1-activating compounds based on benzimidazole and urea-based scaffold has been synthesized.<sup>69,70</sup> However, even if synthetic Sirt1-activating compounds seem to be promising in *in vitro* studies, their clinical efficacy still requires to be tested. The potential therapeutical role of the other members of the sirtuin family still requires to be investigated.

In summary, by participating in transcriptional as well as in metabolic cellular control sirtuins play a crucial role in adaptation to oxidative, genotoxic, and metabolic stress processes all of which increase with aging. In line with this concept, sirtuins should be considered as aging sensors, counteracting deleterious consequences of biological events triggered by aging. Among sirtuins localized in the nucleus, Sirt1 and Sirt6 both play an important role in preserving vascular health and delaying onset of CV disorders, while the role of Sirt7 is still unclear. The mitochondrial sirtuin Sirt3 is involved in mitochondrial homeostasis, playing a protective role in the heart.<sup>71</sup>

## Adaptor protein p66<sup>Shc</sup>

The mitochondrial adaptor protein p66<sup>Shc</sup> is a key determinant of aging; indeed, its genetic deletion in the mouse lowers levels of ROS and prolongs lifespan by 30%.<sup>72</sup> The mammalian SHC locus encodes for three different isoforms with respective molecular weights of 46, 52, and 66 kDa. Due to its unique NH<sub>2</sub>-terminal region, p66<sup>Shc</sup> is the only protein that plays a role in redox metabolism.<sup>72</sup> P66<sup>Shc</sup> regulates ROS production by controlling the partition of ATP generation in the cell and by participating to the electron flow chain in the mitochondria, a major source of cellular ROS, by opening the mitochondrial permeability transition pore (PTP) (Figure 1).<sup>73</sup> In p66<sup>Shc-/-</sup> mice, levels of intracellular ROS are reduced as is oxidative damage of DNA and proteins. Furthermore, their cells are more resistant to paraquat-induced oxidative stress.<sup>72,74</sup>

The pivotal role of p66<sup>Shc</sup> in oxidative stress, together with the fact that p66<sup>Shc</sup> levels increase with aging, makes this adaptor protein a plausible target for age-dependent CV and CBV diseases. The potential role of p66<sup>Shc</sup> is particularly promising in stroke. First, age-dependent endothelial dysfunction of the basilar artery is blunted in aged p66<sup>Shc-/-</sup> mice when compared with age-matched wild type due to reduced ROS production in the former compared with the latter.<sup>75</sup> Secondly, p66<sup>Shc</sup> is crucially involved in endothelial dysfunction induced by hypertension, a major risk factor for stroke. Indeed, exposure of human aortic endothelial cells to cyclic stretch leads via integrin  $\alpha 5 \beta 1$  and the c-Jun N-terminal kinase to a stretch- and time-dependent phosphorylation of p66<sup>Shc</sup> at Ser36. In parallel, nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) is activated and the production of ROS increases,

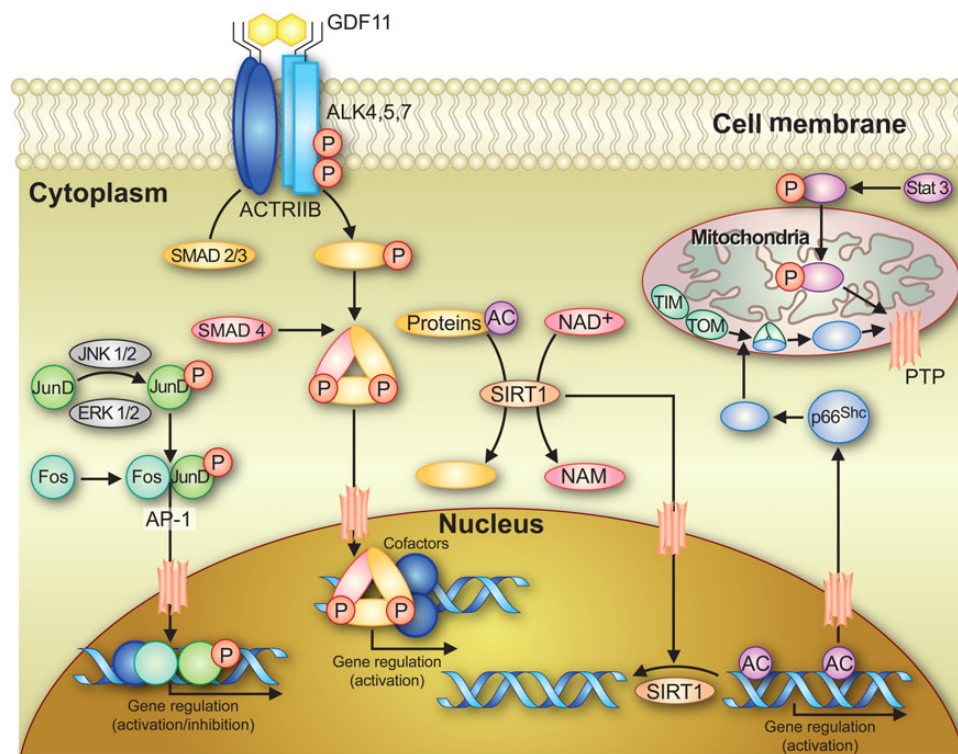
meanwhile NO bioavailability decreases. In this setting, silencing of p66<sup>Shc</sup> blunts stretch-increased O<sub>2</sub><sup>-</sup> production and activation of NADPH oxidase, thus restoring NO bioavailability through a reduced scavenging action of O<sub>2</sub><sup>-</sup>. In line with the above, activation of p66<sup>Shc</sup> is increased in isolated aortic endothelial cells of spontaneously hypertensive compared with normotensive Wistar Kyoto rats.<sup>76</sup> Finally, p66<sup>Shc</sup> knockout mice display decreased production of free radicals in the brain and systemically and have smaller strokes in an ischaemia-reperfusion injury model using middle cerebral artery occlusion. In line with smaller strokes, p66<sup>Shc-/-</sup> mice also exhibit preserved neurological function.<sup>77</sup> Similarly, post-ischaemic *in vivo* silencing of p66<sup>Shc</sup> upon reperfusion improves stroke outcome in wild-type mice while its expression correlates well with short-term outcome in patients with ischaemic stroke (Figure 2).<sup>78</sup> A major mechanism for the reduced stroke size and neurological deficits in p66<sup>Shc-/-</sup> mice and in those in which p66<sup>Shc</sup> was silenced at the time of ischaemia and reperfusion with specific siRNA, appears to be the regulation of proteins such as claudin-5 involved in the permeability of the blood–brain barrier. Indeed, in mice subjected to ischaemia and reperfusion of the middle cerebral artery, p66<sup>Shc</sup> increases in brain oedema and thereby contribute to anexpanding stroke with greater neurological deficits over time and lower survival. Of great clinical importance is the fact that in patients with acute stroke, the expression of p66<sup>Shc</sup> is increased and that this increase is related to their neurological deficit as assessed by the NIH Stroke Score.

P66<sup>Shc</sup> is also involved in the effects of risk factors particularly important in atherosclerosis. Indeed, incubation of human aortic endothelial cells with oxidized low-density lipoprotein (oxLDL) leads to phosphorylation of p66<sup>Shc</sup> at Ser36 that is prevented by inhibition of the lectin-like oxLDL receptor-1 (LOX-1). Silencing of p66<sup>Shc</sup> blunts oxLDL-induced ROS production, underscoring the critical role of p66<sup>Shc</sup> in oxLDL-induced oxidative stress in endothelial cells.<sup>79,80</sup> In line with these *in vitro* findings, p66<sup>Shc-/-</sup>/ApoE<sup>-/-</sup> mice subjected to a high-cholesterol diet exhibit markedly reduced plaque formation suggesting that the adaptor protein facilitates the atherosclerotic process.

Furthermore, p66<sup>Shc</sup> is involved in the vascular and myocardial changes occurring in diabetes mellitus.<sup>81,82</sup> Indeed, in streptozotocin-induced diabetes of the mouse, endothelium dysfunction is markedly attenuated in p66<sup>Shc-/-</sup> mice when compared with their wild-type littermates. Importantly, monocytes obtained from patients with diabetes exhibit enhanced expression of the adaptor protein. Finally, mice with streptozotocin-induced diabetes develop diabetic cardiomyopathy with impaired left ventricular function, a process that is abrogated in p66<sup>Shc-/-</sup> mice by improved function of resident stem cells.

So far most interventions aiming at reducing CV events in diabetics have failed. Therefore, the concept of hyperglycaemic memory has evolved providing an explanation why hyperglycaemia promotes vascular dysfunction even after glucose normalization. Interestingly, in human aortic endothelial cells exposed to high glucose and aortas of diabetic mice, activation of p66<sup>Shc</sup> by protein kinase C  $\beta$ II persists even after re-establishment of normoglycaemia, with continued production of ROS, reduced NO bioavailability, and apoptosis. On the other hand, *in vitro* and *in vivo* gene silencing of p66<sup>Shc</sup>, performed at the time of glucose normalization, suppresses





**Figure 1** Core signalling in JunD-, GDF11-, Sirt1-, and p66<sup>Shc</sup>-mediated pathways. JunD pathway: complex formation of the phosphorylated JunD with the member of the Fos family causes formation and nuclear accumulation of the AP-1 transcription complex which directly regulates gene transcription in association with other cofactors. GDF11 pathway: GDF11-mediated complex formation of the phosphorylated SMAD2/3 with SMAD4 causes nuclear translocation and accumulation of active contraction of Sma and Mad complexes, which directly regulate gene transcription together with other cofactors. Sirt1 pathway: cytoplasmic Sirt1 directly deacetylates different target proteins using nicotinamide adenine dinucleotide as a cofactor, whereas nuclear Sirt1 directly deacetylates key histone residues within DNA-nucleosome complex and thus leads to inactivation of gene transcription. P66<sup>Shc</sup> pathway: complex formation with transporter inner membrane-transporter outer membrane import system causes mitochondria translocation and accumulation of active adaptor protein p66<sup>Shc</sup>, which directly regulate opening of PTP in conjunction with phosphorylated transcription factor Stat3. JNK, c-Jun N-terminal kinases; ERK, extracellular signal-regulated kinases; GDF11, growth differentiation factor 11; ALK, activin receptor-like kinase; ACTR, activin receptors; SMAD, contraction of Sma and Mad (Mothers against decapentaplegic); Sirt1, silent-mating type information regulation 2 homolog 1; NAD<sup>+</sup>, nicotinamide adenine dinucleotide; TIM, transporter inner membrane; TOM, transporter outer membrane; Stat3, signal transducer and activator of transcription 3; PTP, permeability transition pore.

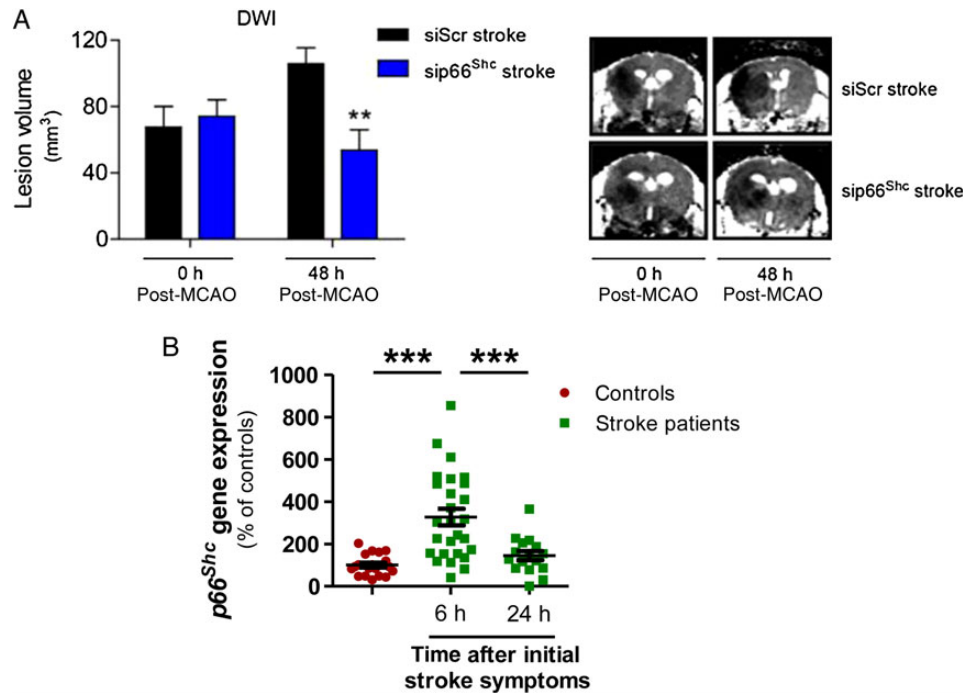
ROS production, restores endothelium-dependent relaxation, and attenuates apoptosis.<sup>83</sup>

Recently, it has been proposed that prolyl isomerase Pin1 plays an important role in the regulation of aging and participates in the mitochondrial translocation of p66<sup>Shc</sup>.<sup>84</sup> Of note, Pin1 inhibition prevents oxidative stress and mitochondrial disruption in cultured human endothelial cells and also in mice under hyperglycaemic conditions.<sup>85</sup>

As p66<sup>Shc</sup> is involved in the effects of most of CV risk factor on the endothelium, it would be expected to play a key role in myocardial infarction as well. Indeed, in peripheral blood monocytes of patients with ACS, RNA levels of p66<sup>Shc</sup> are increased when compared with patients with CAD or normal coronary arteries. Furthermore, p66<sup>Shc</sup> expression is directly related to malondialdehyde levels, a marker of lipid peroxidation and systemic oxidative stress.<sup>86</sup> However, in contrast to these preliminary clinical findings, genetic deletion or *in vivo* silencing of p66<sup>Shc</sup> is associated with larger infarcts after 30 min of occlusion of the left anterior descending artery

followed by 24 h of reperfusion (Figure 3).<sup>87</sup> Of note, this effect was not seen when coronary occlusion time was prolonged to 45 or 60 min. These findings are in contrast with previous findings where perfused murine hearts devoid of p66<sup>Shc</sup> subjected to 40 min of global ischaemia followed by 15 min of reperfusion were significantly protected from ischaemia-reperfusion damage.<sup>88</sup> In light of the above, it is still unclear whether the discrepancies observed are due to *in vivo* vs. *in vitro* preparation or due to the difference in the severity of the insult. Possibly, p66<sup>Shc</sup> plays a different role in the blood vessel wall, in endothelial cells and monocytes than in the myocardium where it appears to play a protective role against ischaemia. Indeed, the damaging effects of p66<sup>Shc</sup> in stroke are entirely related to its effects on endothelial cells since neurons do not express the adaptor protein.

The adaptor protein p66<sup>Shc</sup> has been considered to act as an aging gene based on a study using a limited number of mice.<sup>72</sup> However, a recent study employing a much larger cohort of mice showed no increase in life span in mice with genetic deletion of p66<sup>Shc</sup> pointing



**Figure 2** Impact of p66<sup>Shc</sup> on stroke. (A) Diffusion-weighted imaging denotes reduced lesions in stroke mice silenced *in vivo* post-ischaemically with sip66<sup>Shc</sup> (diffusion-weighted imaging:  $n = 5$ ) compared with siScr stroke mice ( $n = 7$ ) at 48 h post-middle cerebral artery occlusion. Right panel: representative MRI images. Data are expressed as mean  $\pm$  SEM; \*\* $P < 0.01$  for sip66<sup>Shc</sup> stroke vs. siScr stroke. (B) P66<sup>Shc</sup> gene expression in peripheral blood monocytes from ischaemic stroke patients. Real-time PCR determined increased p66<sup>Shc</sup> mRNA levels in stroke patients 6 h ( $n = 27$ ), but not 24 h ( $n = 16$ ) after initial stroke symptoms compared with the levels of control subjects ( $n = 19$ ). Data are expressed as mean  $\pm$  SEM; \*\*\* $P < 0.001$  (adapted from Spescha *et al.* <sup>78</sup>).

out that perhaps the role of p66<sup>Shc</sup> in determining lifespan is not yet clear.<sup>89</sup> Additionally, the same study also demonstrated the role of p66<sup>Shc</sup> on several disease processes including insulin signalling, stress resistance, and energy metabolism. Therefore, additional research is required in order to fully understand the extent of p66<sup>Shc</sup> influence on lifespan and age-related changes under physiological and pathophysiological conditions.

Nonetheless, the above-described data address the aging gene p66<sup>Shc</sup> as a valuable therapeutic target in endothelial dysfunction and vascular disease, whereas its role in myocardial infarction still requires further investigation.

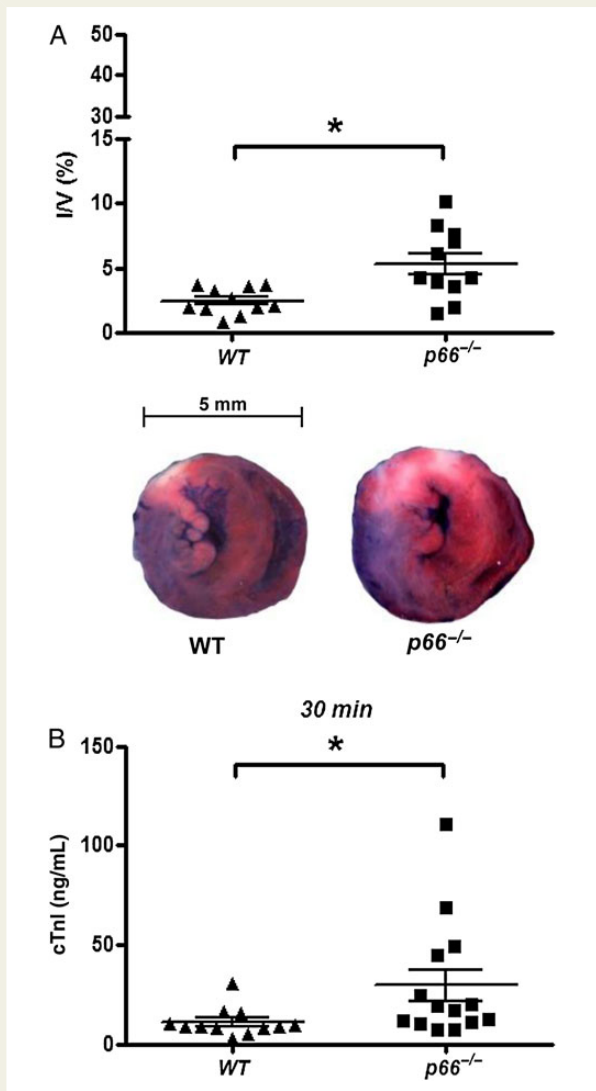
## The transcription factor JunD

JunD is the last discovery in Jun family of activator protein-1 transcription factor complex. In contrast with two other members of Jun family, c-jun and JunB, JunD shows different gene regulation profile and function (Figure 1).<sup>90</sup> Interestingly, mice with genetic deletion of JunD are viable, whereas the deletion of either c-jun or JunB is embryonically lethal.<sup>91</sup> JunD can act as positive or negative regulator of transcription of a broad variety of cell-type-specific genes involved in oxidative stress, cell proliferation, and differentiation.<sup>92–94</sup> Experimental data over the last decade have shown the protective role of JunD against oxidative stress by modulating the expression of several free radical scavenging enzymes.<sup>95</sup> In addition, recent evidence was obtained pointing out that JunD may

protect from aging-induced endothelial dysfunction and thus may be considered as a novel target to prevent ROS-mediated vascular aging (Figure 4).<sup>96</sup> Both young and old JunD knockout mice showed impaired endothelium-dependent relaxation when compared with age-matched wild-type control mice. Endothelial dysfunction was associated with age-independent decrease in endothelial NO release, eNOS activity, and increase in mitochondrial O<sub>2</sub><sup>•−</sup> generation and ONOO<sup>−</sup> levels. In contrast, JunD *in vivo* overexpression restored endothelial function.<sup>96</sup> Of note, JunD expression was significantly reduced in peripheral blood monocytes of old healthy subjects and was correlated with the expression of scavenging and oxidative enzymes.<sup>96</sup> Finally, JunD expression was decreased in heart failure patients suggesting that it may be protective from aging-related cardiac dysfunction.<sup>97,98</sup>

## Growth differentiation factor 11

Growth differentiation factor 11 is a member of TGF $\beta$  superfamily with homology to myostatin (Figure 1). Interestingly, in mice its levels decline with age, whereas myostatin and TGF $\beta$ 1 levels remain unchanged, suggesting a role of this molecule in aging process.<sup>99</sup> Of note, GDF11 is involved in cardiac hypertrophy. In line with this, old mice exposed for 4 weeks to the blood circulation of young mice by heterochronic parabiosis—a surgical model by which the circulatory system of two mice, a young one and an old one, are joined together so as to have a single circulatory system—cardiac hypertrophy



**Figure 3** Genetic deletion of  $p66^{Shc}$  leads to larger infarcts. (A) Quantification of infarct size (I) per V with the representative images of 2-3-5-triphenyl tetrazolium chloride-stained middle heart sections of control wild-type and  $p66^{-/-}$  mice after 30 min of ischaemia followed by 24 h of reperfusion.  $n = 11$  for wild type and  $n = 14$  for  $p66^{-/-}$ . (B) Serum cardiac troponin I (cTnI) levels of WT or  $p66^{-/-}$  after ischaemia and reperfusion injury.  $n = 10$  for both genotypes. Data are mean  $\pm$  SEM;  $*P < 0.05$  vs. wild type (adapted from Akhmedov et al.<sup>87</sup>).

dramatically regressed, along with a reduced cardiomyocyte size and molecular remodelling. Growth differentiation factor 11 is a determinant of this phenomenon, since treating old mice with GDF11 to reach youthful levels reproduced the effects of parabiosis and also reversed age-related cardiac hypertrophy.<sup>100</sup>

Furthermore, GDF11 also shows therapeutic potential in age-related neurodegenerative and neurovascular diseases. In fact, after 5 weeks of exposure to the circulation of young mice by heterochronic parabiosis old mice exhibited active cerebral vascular remodelling, culminating in increased neurogenesis. These effects were reproduced by treating old mice with daily injections of

GDF11 in order to reach the circulating concentrations of GDF11 of young mice.<sup>101</sup> Although the current evidences on GDF11 are still limited, the above-reported data indicate a potential key role of GDF11 in reversing several pathological processes associated with aging and, consequently, with age-related CV and CBV diseases.

## Clinical perspectives

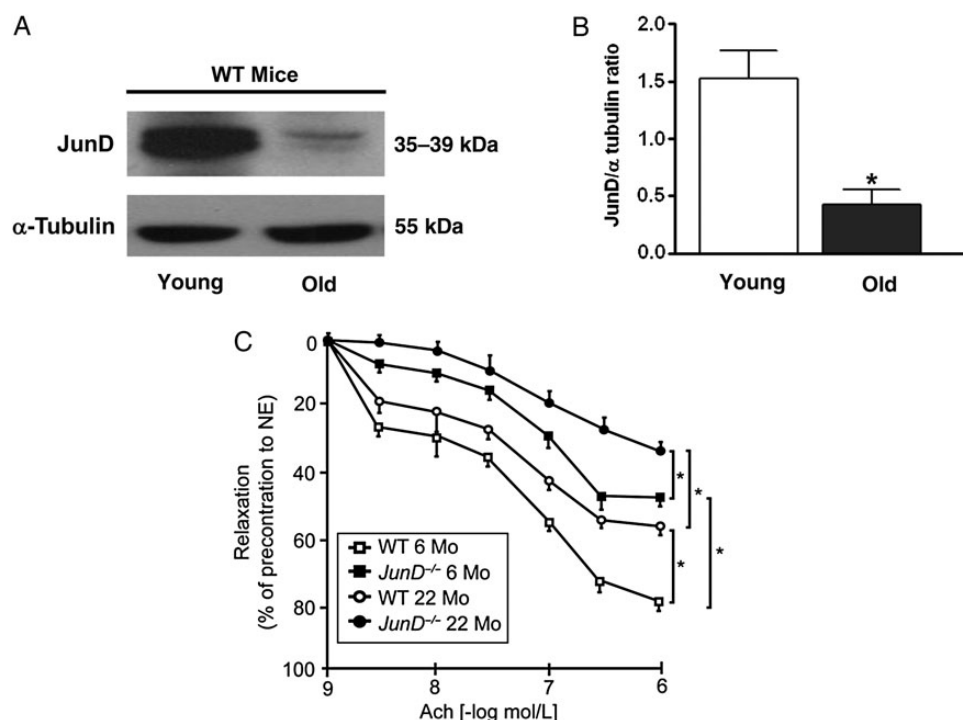
### Life style and caloric restriction

The relationship between lifestyle, dietary factors, and CV/CBV diseases has been a focus of scientific research for almost half century. Interestingly, physical training, a life style measure known to reduce CV risk, increases the activity of Sirt1 by enhancing nicotinamide phosphoribosyltransferase and thereby the production of sirtuin-fuelling  $NAD^+$ . Additionally, exercise training normalizes the age-associated shift in redox balance, since trained animals have lower levels of carbonylated proteins, reduced expression of hypoxia-inducible factor-1 $\alpha$  and of vascular endothelial growth factor. Furthermore, also the age-associated increase in the level of Sirt6 is attenuated by exercise training. While data on other aging and longevity pathways are still missing, it appears that regular exercise decelerates the effects of the aging process via sirtuin-dependent pathways.<sup>102</sup>

Resveratrol (3,4',5-trihydroxystilbene), a small polyphenol found in various berries, nuts, grapes, wine, and other plants sources, might be more promising for its anti-inflammatory, antioxidant, and anti-aging effects.<sup>103</sup> It is a natural activator of Sirt1 and binds the Sirt1 enzyme-peptide substrate complex at an allosteric site amino-terminal to the catalytic domain thus lowering the Michaelis constant for acetylated substrates.<sup>68</sup> In line with this and as Sirt1 is involved in the regulation of glucose metabolism, in diet-induced obese and genetically obese mice, resveratrol improves insulin sensitivity, lowers plasma glucose, and increases mitochondrial capacity.<sup>58</sup> Additionally, in rats, resveratrol improves whole-body glucose homeostasis and insulin sensitivity in adipose tissue, skeletal muscle, and liver.<sup>68</sup> However, these promising findings obtained in preclinical studies were not completely confirmed at the clinical level where results have been contrasting.<sup>104,105</sup> In particular, 8-week administration of resveratrol did not reduce insulin sensitivity, liver steatosis, or abdominal fat distribution in overweight or obese individuals with clinically established non-alcoholic fatty disease.<sup>105</sup> Similarly, the benefits induced by resveratrol in reducing atherosclerosis progression in  $ApoE^{-/-}$  mice were not completely confirmed in patients.<sup>106,107</sup>

Caloric restriction (CR) is a dietary regimen that improves health and slows aging by limiting dietary energy intake. In different species from yeast to mammals, CR delays the onset of age-associated diseases, including cancer, atherosclerosis, and diabetes.<sup>108</sup> Decreased systemic oxidative damage has been proposed as a mechanism and increasing evidence suggests that Sirt1 could play a key role in mediating the benefits induced by CR.<sup>109</sup> In mice maintained for 3 months on CR, the amount of mitochondrial DNA, expression of peroxisome proliferator-activated receptor- $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ), nuclear respiratory factor-1, mitochondrial transcription factor A (Tfam), expression of cytochrome c oxidase (COX-IV), and cytochrome c (Cyt c), that are two mitochondrial proteins involved





**Figure 4** Role of JunD in vascular aging. (A and B) Representative western blot (A) and densitometric quantification (B) of JunD protein expression from aortic lysates of young and old wild-type mice. (C) Age-dependent changes in endothelium-dependent relaxation in aortic rings from wild-type and *JunD*<sup>-/-</sup> mice. Line graphs show concentration–response curves to acetylcholine. Results are presented as mean  $\pm$  SEM;  $n = 5–7$  per group. NE, norepinephrine. \* $P < 0.05$  (adapted from Paneni et al.<sup>96</sup>).

in cell respiration, oxygen consumption, and expression of mitofusin 1 and 2 was higher in white adipose tissue and other tissues compared with mice fed a normal diet, suggesting a role of CR in improving mitochondrial function. These processes were paralleled by an increased eNOS and Sirt1 expression that could explain the benefits induced by CR on lifespan and age-related CV and CBV diseases.<sup>108</sup>

In monkeys assigned to CR, individually determined baseline intake was reduced by 10% per month over a 3-month period to reach the desired 30% restriction. Animals were fed a semi-purified, nutritionally fortified, low-fat diet containing 15% protein and 10% fat. Monkeys undergoing CR have shown an improved age-related and all-cause mortality when compared with control animals.<sup>110</sup> Additionally, CR was shown to have no impact on non-age-related deaths.<sup>110</sup>

In mice assigned to CR, consisting in a 30% restriction of caloric intake for 7 days, infarct size following permanent coronary ligation was reduced when compared with mice assigned to an *ad libitum* diet. Additionally, CR mice showed higher ejection fraction and dP/dt max as well as smaller end-systolic volume when compared with mice fed *ad libitum* at 2 days post-myocardial infarction. Caloric restriction diet was associated with increased levels of phosphorylation of Akt and GSK3 $\beta$ , reduced levels of phosphorylated AMPK and mitochondrial-related proteins PGC-1 $\alpha$ , cytochrome c, and cyclooxygenase IV, with no differences in the levels of phosphorylated eNOS or MAPK (ERK1/2; p38) and reduced protein abundance of cleaved caspase3 in the infarcted heart.<sup>111</sup>

Caloric restriction was demonstrated to impact also cardiac remodelling. Indeed, in DS/obese rats (with metabolic syndrome and hypertension), 35% CR for 4 weeks reduced body fat content, ameliorated left ventricular hypertrophy, fibrosis, diastolic dysfunction, and attenuated cardiac oxidative stress and inflammation when compared with rats fed *ad libitum*.<sup>112</sup> Similarly, in mice assigned to CR, consisting in a 40% restriction of caloric intake for 4 weeks, morphological and functional changes (left ventricular hypertrophy, impaired left ventricular relaxation) following ascending aortic constriction were less evident when compared with mice fed *ad libitum*.<sup>113</sup>

The impact of CR on the key features of arterial aging has also been investigated. In mice, life-long 40% CR blunted the increase in blood pressure, arterial stiffness, and carotid wall thickness induced by aging when compared with mice fed *ad libitum*. Similarly, CR attenuated nitrotyrosine and superoxide production blunted the increase in NADPH activity and p67 expression resulting in an increase of SOD and an enhanced NO bioavailability thus preventing age-induced endothelial dysfunction.<sup>114</sup>

The importance of specific diet regimens and CR in reducing CV risk has been demonstrated also in humans. The INTERHEART study confirmed the potential protective role of fruit and vegetable consumption on the risk of developing myocardial infarction,<sup>115</sup> whereas in the PREDIMED study Mediterranean diet supplemented with extra-virgin oil or with mixed nuts reduced the risk of major CV events by 30% when with a traditional low-fat diet,<sup>116</sup> supporting

the hypothesis that specific diet regimens can reduce CV risk. The benefits induced by CR in humans have been also directly demonstrated by an additional study whereby 18 subjects who had been on CR for an average of 6 years were age-matched to 18 healthy individuals on typical American diets. Serum total cholesterol, LDL cholesterol, ratio of total cholesterol to HDL cholesterol, triglycerides, fasting glucose, fasting insulin, CRP, PDFG-AB, and systolic and diastolic blood pressure were all markedly lower, whereas HDL-C was higher, in the CR than in the American diet group. Additionally, carotid artery IMT was  $\sim 40\%$  less in the CR group than in the comparison group.<sup>117</sup> In the CALERIE trial, participants were randomized to 1 of the following 4 groups for 6 months: control (weight maintenance diet); CR (25% CR of baseline energy requirements); CR with exercise (12.5% CR plus 12.5% increase in energy expenditure by structured exercise); very low-calorie diet (890 kcal/day until 15% weight reduction, followed by a weight maintenance diet). At 6 months, fasting insulin levels were significantly reduced from baseline in the intervention groups, whereas DHEAS and glucose levels were unchanged. Core body temperature was reduced in the CR and CR with exercise groups. Sedentary 24-h energy expenditure was decreased in the CR, CR with exercise, and very low-calorie diet groups, but not in controls.<sup>118</sup> Finally, in another study enrolling 25 subjects practicing CR for  $6.5 \pm 4.6$  years and 25 age- and gender-matched control subjects consuming Western diets, standard transmitral Doppler flow diastolic function indexes of the CR group were similar to those of younger individuals, and model-based image processing, flow-derived diastolic function indexes, reflecting chamber viscoelasticity and stiffness, were significantly lower than in control subjects. Additionally, blood pressure, serum C reactive protein, TNF- $\alpha$ , and TGF- $\beta 1$  levels were significantly lower in the CR group compared with the Western diet group, demonstrating that CR has cardiac-specific effects that ameliorate aging-associated changes in diastolic function<sup>119</sup> (refer to Table 1 for a summary of all recent basic research and clinical studies).

## Arterial stiffness

Age is an important determinant of pulse wave velocity.<sup>120</sup> The wall of large arteries, in particular the aorta, becomes thicker and less elastic with age, resulting in an increased pulse wave velocity, an important parameter of arterial stiffness.<sup>140</sup> In general, arterial stiffness reflects gradual fragmentation and loss of elastin fibres with simultaneous accumulation of stiffer collagen fibres in the media layer of large arteries and normally occurs independently of any other processes. Because arterial compliance is determined as a ratio of collagen to elastin, aging is associated with a decrease of this ratio due to accelerated degradation of elastin and accumulation of stiffer collagen. Such elastin degradation is further associated with progression in aortic stiffness and mortality.<sup>121</sup> Increased arterial stiffness reduces the reservoir function of the affected arteries and increase pulse wave velocity, thus increasing systolic and pulse pressure. Arterial stiffening is considered to be a predictor of several CV outcomes, such as stroke, myocardial infarction, and kidney diseases.<sup>122–124</sup> In a recently published Framingham Heart Study Offspring cohort, it has been shown that increased aortic stiffness was associated with the higher risk of incident hypertension<sup>125</sup> (refer to Table 1 for a summary of all recent basic research and clinical studies).

## Pharmacological interventions

Recently, several cohort studies or randomized controlled trials attempted to assess the benefits provided by food additives assumed to decrease oxidative stress in large populations. Vitamins, due to their antioxidant properties *in vitro*, are of particular interest in this context and, indeed, in a first study daily vitamin E intake of  $>100$  IU reduced atherosclerosis progression and event rates.<sup>126,127</sup> However, the hypothesis that vitamin E supplementation could reduce atherosclerosis progression and clinical events was not confirmed by later studies. In the Vitamin E Atherosclerosis Prevention Study,<sup>128</sup> 353 subjects older than 40 years, with high LDL cholesterol levels and no clinical signs or symptoms for CV disease were randomized to DL-  $\alpha$ -tocopherol 400 IU per day, or placebo and followed every 3 months for an average of 3 years. Compared with placebo,  $\alpha$ -tocopherol supplementation significantly raised plasma vitamin E levels, reduced circulating oxidized LDL and reduced LDL oxidative susceptibility, but did not influence the progression of carotid intima-media thickness over a 3-year period. In another study, the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study,<sup>129</sup> men with a previous myocardial infarction randomized to alpha-tocopherol displayed a significant reduction in non-fatal myocardial infarction, but at the same time, a more relevant increase in fatal CAD risk; similarly subjects receiving the combination of  $\alpha$ -tocopherol and  $\beta$ -carotene exhibited an increase in fatal CAD. In subjects without history of myocardial infarction enrolled in the same study  $\alpha$ -tocopherol alone or in combination with  $\beta$ -carotene did not affect fatal CAD nor non-fatal myocardial infarction risk. Possibly, vitamin E supplementation suppresses cellular antioxidant systems (as it was demonstrated for ubiquinol-10)<sup>130</sup> or does not reach high enough levels in cellular compartments to exert its antioxidant effects. Furthermore, it is conceivable that vitamin E may become pro-oxidant in the absence of sufficient levels of other antioxidants. Finally, in the HOPE trial a combination of vitamins did not affect clinical outcome; indeed, in a subset it increased the incidence of heart failure.<sup>131</sup> Thus, reducing ROS levels using exogenous antioxidants such as vitamins does not induce any benefit in terms of reduction of CV/CBV risk.

Angiotensin-converting enzyme (ACE) could represent an additional interesting target for pharmacological intervention; indeed, ACE converts biologically inactive angiotensin I (Ang I) into active angiotensin II (Ang II), a potent vasoconstrictor, and its inhibitors are already an established therapeutical principle for the treatment of hypertension. Activation of vascular ACE may also influence VSMC growth and oxidative state of the vessel wall.<sup>141</sup> In addition, production of ROS (superoxide and hydrogen peroxide) enhanced by Ang II, has been associated with vascular inflammation, atherosclerosis, hypertrophy, remodelling and angiogenesis,<sup>132,142</sup> all of which represent important components of age-dependent vascular dysfunction. Angiotensin-converting enzyme inhibitors block activation of renin-angiotensin system and slow down the progression of heart failure and atherosclerosis.<sup>132,142</sup> Several clinical trials with ACE inhibitors also showed reduced overall mortality and prolonged life expectancy in patients suffering from myocardial infarction.<sup>133–138</sup> Other large-scale trials, in particular CAPP with captopril, HOPE with ramipril, SOLVD with enalapril and PEACE with trandolapril, showed reduced incidence in *de novo* diabetes connected to ACE-inhibitor therapy.<sup>138,139,143,144</sup>

**Table 1** Overview of recent clinical and basic research studies focusing on vascular aging

Study	Findings	
Koltai <i>et al.</i> <sup>102</sup>	Physical training in rats	Increases Sirt1 activity by enhancing NAMPT and Sirt-fueling NAD <sup>+</sup> Normalizes the age-associated shift in redox balance Attenuates the age-associated increase in the levels of Sirt6
Banks <i>et al.</i> <sup>58</sup>	Resveratrol in diet-induced obese and genetically obese mice	Improves insulin sensitivity Lowers plasma glucose Increases mitochondrial capacity
Milne <i>et al.</i> <sup>68</sup>	Resveratrol in rats	Improves whole-body glucose homeostasis Improves insulin sensitivity in adipose tissue, skeletal muscle, and liver
Méndez-del Villar <i>et al.</i> <sup>104</sup>	Resveratrol in patients with metabolic syndrome	Decreases weight, BMI, waist circumference, and fat mass Decreases insulin secretion
Chachay <i>et al.</i> <sup>105</sup>	Resveratrol in overweight or obese individuals with clinically established non-alcoholic fatty disease	Does not reduce insulin sensitivity Does not reduce liver steatosis Does not reduce abdominal fat distribution
Norata <i>et al.</i> <sup>106</sup>	Resveratrol in ApoE <sup>-/-</sup> mice	Reduces the presence of atherosclerotic plaque by 40 and 36% in the aortic sinus and in the ascending aorta, respectively
Sahebkar <i>et al.</i> <sup>107</sup>	Resveratrol in humans	Does not affect lipid plasma levels
Nisoli <i>et al.</i> <sup>109</sup>	Caloric restriction in males	Induces endothelial nitric oxide synthase expression Induces 3',5'-cyclic guanosine monophosphate formation Induces mitochondrial biogenesis Induces oxygen consumption Induces adenosine triphosphate production Induces expression of Sirt1
Colman <i>et al.</i> <sup>110</sup>	Caloric restriction in monkeys	Improves age-related and all-cause mortality
Noyan <i>et al.</i> <sup>111</sup>	Caloric restriction in mice	Reduces infarct size following permanent coronary ligation Determinates higher ejection fraction when compared with controls Determinates higher dp/dt max values when compared with controls Determinates smaller end-systolic volume as compared with controls
Takatsu <i>et al.</i> <sup>112</sup>	Caloric restriction in mice with metabolic syndrome and hypertension	Reduces body fat content Ameliorates left ventricular hypertrophy Ameliorates fibrosis Ameliorates diastolic dysfunction Attenuates cardiac oxidative stress and inflammation
Kobara <i>et al.</i> <sup>113</sup>	Caloric restriction in mice	Attenuates morphological and functional changes following ascending aortic constriction
Donato <i>et al.</i> <sup>114</sup>	Caloric restriction in mice	Blunts the increase in blood pressure induced by aging Blunts the increase in arterial stiffness induced by aging Blunts the increase in carotid wall thickness induced by aging Attenuates oxidative stress Enhances endothelial function
INTERHEART study <sup>115</sup>	Fruit/vegetable consumption in humans	Reduces risk of myocardial infarction
PREDIMED study <sup>116</sup>	Mediterranean diet in humans	Reduces the risk of major CV events
Fontana <i>et al.</i> <sup>117</sup>	Caloric restriction in humans	Reduces serum total cholesterol, LDL cholesterol, ratio of total cholesterol to HDL cholesterol, triglycerides Reduces fasting glucose, fasting insulin Reduces CRP Reduces blood pressure Increases HDL cholesterol
CALERIE study <sup>118</sup>	Caloric restriction in humans	Reduces fasting insulin levels Reduces core body temperature and sedentary 24h energy expenditure
Meyer <i>et al.</i> <sup>119</sup>	Caloric restriction in humans	Ameliorates aging-associated changes in diastolic function
AlGhatrif <i>et al.</i> <sup>120</sup>	Arterial Stiffness in humans	Increases with age and is sex dependent
Smith <i>et al.</i> <sup>121</sup>	Arterial Stiffness in humans	Associated with elastin degradation and overall mortality

Continued

**Table 1** Continued

Study	Findings	
Karras et al. <sup>122</sup>	Arterial Stiffness in humans	Associated with all-cause mortality and CV events in chronic kidney disease
Hashimoto et al. <sup>123</sup>	Arterial Stiffness in humans	Linked to an increased risk of stroke in hypertension
Kitzman et al. <sup>124</sup>	Arterial Stiffness in humans	Contributes to exercise intolerance in heart failure patients
Kaess et al. <sup>125</sup>	Arterial Stiffness in humans	Associated with higher risk of incident hypertension, but not with initial blood pressure
Stempfer et al. <sup>126</sup>	Vitamin E supplements in middle-aged women	Are associated with a reduced risk of coronary heart disease
Rimm et al. <sup>127</sup>	High consumption of vitamin E in men	Is associated with a lower risk of coronary heart disease
VEAPS <sup>128</sup>	Vitamin E supplements in humans	Reduces circulating oxidized LDL Reduces LDL oxidative susceptibility Does not reduce the progression of IMT
Alpha-Tocopherol Beta-Carotene Cancer Prevention study <sup>129</sup>	Alpha-tocopherol or beta-carotene supplements in humans	Do not reduce major CV events Increase the risk of fatal coronary heart disease
HOPE trial (vitamin E sub-analysis) <sup>130,131</sup>	Long-term vitamin E supplementation	Does not prevent cancer Does not prevent major CV events May increase the risk of heart failure
SOLVD trial <sup>132</sup>	Enalapril in patients with asymptomatic left ventricular dysfunction	Reduces the incidence of heart failure and the rate of related hospitalizations Reduces the incidence of diabetes, especially in presence of impaired fasting plasma glucose
GISSI-3 trial <sup>133</sup>	6 months Lisinopril treatment in patients with acute myocardial infarction within 24 h of onset of symptoms	Reduces the risk of death or severe ventricular dysfunction
ISIS-4 trial <sup>134</sup>	1 month captopril therapy	Reduces the risk of mortality
SAVE trial <sup>135</sup>	Captopril in patients after an acute myocardial infarction and with left ventricular dysfunction without overt heart failure	Reduces the risk of mortality Reduces the risk of fatal and non-fatal CV events Reduces the risk of developing severe heart failure Reduces the risk of heart failure hospitalization
EUROPA trial <sup>136</sup>	Perindopril in patients with previous myocardial infarction or coronary artery disease	Reduces the risk of CV death or myocardial infarction or cardiac arrest
HOPE trial <sup>137</sup>	Ramipril in high risk patients without heart failure	Reduces the risk of myocardial infarction or stroke or CV death Reduces the risk of CV death Reduces the risk of myocardial infarction Reduces the risk of stroke Reduces the risk of all-cause death Reduces the risk of revascularization procedures Reduces the risk of cardiac arrest Reduces the risk of heart failure Reduces the risk of complications related to diabetes
CAPP trial <sup>138</sup>	Captopril in patients with hypertension	Reduces the risk of fatal and non-fatal myocardial infarction, stroke, and other CV death Reduces CV mortality Reduces the risk of fatal and non-fatal myocardial infarction Reduces the risk of new onset of diabetes mellitus
PEACE trial <sup>139</sup>	Trandolapril in patients with stable coronary artery disease and normal or slightly reduced left ventricular function	Reduces the risk of CV death or myocardial infarction or coronary revascularization Reduces the risk of diabetes

Simultaneous intervention on multiple targets using multiple pharmacological agents holds several promises which deserve additional investigation. Additionally, discovery of novel therapeutical targets, such as ROS-producing enzymes, angiotensin receptor subtypes, and specific metabolic pathways will likely represent major areas of research in the years to come. Finally, the use of modern molecular tools, such as antisense gene therapy or blocking

antibody will certainly be implemented (refer to Table 1 for a summary of all recent basic research and clinical studies).

## Conclusions

Sirtuins, p66<sup>Shc</sup>, JunD, and GDF11 have all been demonstrated to play a key role in aging and age-related CV and CBV diseases;

thus, they could represent important therapeutical targets to face the ongoing aging pandemic.

Sirtuins are involved in several processes, such as ROS production, apoptosis, and metabolism. Resveratrol, a polyphenolic Sirt1-activating compound found in grapes and wine has been demonstrated to exert several beneficial effects on CV and CBV diseases in animal and *in vitro* models but its effects in humans are still unclear. Physical exercise has been shown to exert beneficial effects on aging and CV and CBV diseases through Sirt1-dependent pathway.

JunD as a distinct member of the Jun family is implicated in aging. Showing a unique regulation and expression profile, it can activate or repress the transcription of a broad collection of target genes involved in oxidative stress, cell growth, and differentiation. Recent research has provided a number of evidences for a protective role of JunD in aging-induced endothelial dysfunction thus representing a selectable novel target to prevent age-related CV and CBV diseases.

P66<sup>Shc</sup> exerts its effects on aging and CV and CBV diseases preventing ROS-mediated cell damage and, consequently, reducing cellular apoptosis. Although, several clinical trials have shown that reducing oxidative stress by antioxidant treatments is not the key for treating age-related diseases, one hypothesis to explain these findings is that the inhibition of ROS production (i.e. decreasing p66<sup>Shc</sup> or increasing Sirt1 activity), but not the reduction of circulating ROS levels once they have been already produced, plays a key role in reducing age-related diseases.

Studies on GDF11 reported surprising results about the efficacy of this factor in aging and few but significant evidences have shown that GDF11 could represent a valuable target for CV and CBV diseases.

The herein described target proteins offer insights to better characterize underlying mechanisms of CV and CBV diseases in aging; however, additional work needs to be done to elucidate their possible interactions and to finalize the characterization of their molecular pathways.

## Future perspectives

To date, much is known to the research community about CV and CBV aging as well as the molecules and molecular mechanisms involved in this process. However, the complex interplay between these mediators is far from being elucidated. A better understanding of the molecular and cellular mechanisms underlying vascular aging, as well as their potential interactions, will provide a list of candidate molecules which could be considered as targets for specific interventions aimed at delaying vascular aging. Oxidative stress together with inflammation is among those mechanisms which could be considered as targets for future treatment or for the development of novel and reliable biomarkers to improve the understanding of risk factor stratification. Finally, the individual molecules discussed in details in this review could set the basis for additional studies aimed at finding possible targets for therapeutical intervention to delay the onset vascular aging.

## Authors' contributions

G.G.C.: handled funding and supervision, acquired the data, conceived and designed the research, drafted the manuscript, and

made critical revision of the manuscript for key intellectual content.

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